Modulation of microbial community permissiveness towards broad host range conjugative plasmid under metal stress

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Results to date suggest we are directly impacting evolution of environmental bacteria, increasing resistance to QACs. Further investigation of the epimerase-containing clones and complete sequencing of unique inserts is currently underway to determine if co-selection was occurring.

1. Kazimierczak, K.A., et al., *Tetracycline resistome of the organic pig gut.* Appl Environ Microbiol, 2009. 75(6): p. 1717-22.

P EVO 6

Multicenter study of resistance to antibiotics in *Enterobacter cloacae*

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Method: Enterobacter cloacae is a major pathogen responsible of nosocomial infections. Pathogénicité is exacerbated by its resistance to antibiotics, acquired by extended spectrum β-lactamases (ESBL) and plasmid AmpC (pAmpC), often associated with resistance to aminoglycosides and quinolones. A multicenter retrospective cohort study was carried out to gain baseline information on antibiotics resistance of E.cloacae in tree hospitals in the west of Algeria.
Result: 158 strains were isolated between September 2009 and May 2012 from various units in the hospitals of Tlemcen, Sidi Bel Abbes and Oran. The analysis of resistance phenotypes to b-lactam has detected diversity phenotypic with dominance of strain producing extended spectrum b-lactamase (ESBL) or 51.3%. The pulsed field gel electrophoresis (PFGE) showed different clonal groups and confirm the epidemic nature of the strains studied.The most isolates produced ESBL CTX-M type, whereas only 5 produced SHV-type ESBLs. The bla_{TEM} gene was found in all strains of *E.cloacae*. One isolate was found to produce plasmid-mediated AmpC b-lactamases (CMY-2), this gene was transferred from *E.*cloacae by eletroporation. Conjugation experiments showed that bla_{CTX-M}, bla_{TEM}, and bla_{SHV} were carried by conjugative plasmids of high molecular weight (≥ 70 kb).
Conclusion: These results show that the frequency of these multiresistant bacteria increasing dramatically in our hospitals and their emergence represents a serious therapeutic and epidemiological problem, hence the need for the establishment of a monitoring system of the microbial environment and strict application of hygiene measures.

P EVO 7

Co-selection for antibiotic resistant bacteria at sub-inhibitory concentration of biocides

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Antibiotic resistance (AR) poses an increasing threat to health care and to the environment. Gaining insights into the mechanisms of selection and enrichment of antibiotic resistant bacteria (ARB) in the gut of humans/animals and the natural environment is essential to tackle the problem. In addition to the use of antibiotics, excessive use of biocides has been hypothesised to result in co-selection for ARB due to genetic linkage between antibiotic and biocide resistance genes. To test this hypothesis, an isogenic pair of bacteria with and without biocide resistance genes, qacE or qacH that are commonly found in ARB, were constructed in low copy number plasmid under control of their natural promoters. The pair was competed in growth medium for 60 generations at sub-inhibitory concentrations of a biocide, benzalkonium chloride (BKC). In addition, sewage influent natural bacterial community was inoculated into medium and allowed to grow for 60 generations in the presence of BKC at sub-inhibitory concentrations. The proportion of the resistance strain to the isogenic susceptible strain and prevalence of class 1 integrons (Int1) in the complex community were quantified using Q-PCR. Minimal inhibitory concentration (MIC) and selective concentration (MSC) of BKC for the resistant strain were determined. It was shown that the biocide selected for qacE or qacH bearing bacteria at sub-inhibitory concentration in the dual strains system and enriched for Int1 bearing bacteria in the semi-natural system. The results indicated that biocides co-selects for ARB under laboratory conditions at sub MIC concentrations and suggests that such co-selection may occur in the natural environment.

P EVO 8

Modulation of microbial community permissiveness towards broad host range conjugal plasmid is metal specific

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Questions: The extent by which antibiotic resistance encoding conjugal plasmids transfer in microbial communities is of acute relevance in the age of massive antibiotic usage. When a plasmid newly enters a bacterial community, the community permissiveness towards plasmids is the key parameter to assess its spread. Transfer and maintenance of plasmids within the community are determined by specific genetic traits of the plasmid and of the donor and recipient strains. Apart from these genetic determinants, the occurrence of stressors might play a major role in altering the acute permissiveness of a bacterial community,

since plasmid transfer is considered a main process in immediate stress response and adaptation to environmental changes. One of the major sources of stress for soil communities is the introduction of metals through geological or anthropogenic sources like manure. We, therefore, aimed to answer the following questions: Does metal stress alter a soil community's permissiveness towards broad host range plasmids and if so, can these changes be explained through the change in community diversity caused by the imposed stress? Do we observe a general stress response with regard to plasmid transfer for different metals introduced at equal inhibition levels or is this stress response metal specific?
Methods: We used a radiolabelled [3H]leucine incorporation approach to measure the 20% and 50% inhibition concentrations for 5 selected metals (Cu, Zn, Ni, Cd, As). A mCherry-tagged red fluorescent *Escherichia coli* donor strain carrying the *gfp*-tagged broad host range plasmid pKJK5 was then mixed with a soil bacterial community and exposed to the metal stressor in a filter mating assay mimicking natural nutrient conditions, with maximized cell-to-cell contact. Plasmid transfer was observed and quantified by detecting green fluorescent transconjugant microcolonies using confocal laser scanning microscopy. Transconjugants were isolated using fluorescent activated cell sorting for bacterial size, gfp-based green fluorescence and exclusion of red fluorescence. Sorted transconjugants and stressed bacterial communities were subsequently analyzed by 16S rRNA gene amplicon pyrosequencing.
Results: The imposed metal stress lowered the plasmid transfer frequency in the filter mating assays. The intensity of this effect was metal specific and could not be explained by the measured growth inhibition of the recipient community because the exposure was normalized to cause similar inhibition. Cadmium had a strong negative effect on the community's permissiveness, while Zinc caused almost no detectable effect.
A high diversity of transconjugants was retained within all transconjugal pools irrespective of stress exposure. Yet results revealed an effect on community permissiveness for the heavy metals Nickel or Copper, while Arsenic exposure caused no effect. The changes in transconjugal pool diversity could not be directly correlated to a change in community diversity through the introduced stress.
Conclusions: We demonstrate that metal stress changes the plasmid transfer ability within soil bacterial communities. Our results furthermore suggest that this effect is not general but corresponds to a metal specific stress response of the soil microbial community with regard to plasmid transfer.

P EVO 9
The role of the insertion sequence IS*256* in genetic flexibility in *Staphylococcus aureus*
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Staphylococcus aureus is a pathogen that causes nosocomial and community-acquired infections. In recent years *S. aureus* has acquired resistance to nearly all antibiotics used in clinical practice. As a component of transposons, insertion (IS) elements are involved in the transfer of resistance genes between strains and species. Furthermore, the integration of a single IS element into a gene or its promoter may result in an inactivation or overexpression of the affected gene. IS*256* is found frequently in CC8 and ST239 strains of *S. aureus*. As exposure to subinhibitory concentrations of antibiotics will not only select for resistant bacteria but may lead to an activation of mutational mechanisms, as for example the SOS response (1), the following questions were addressed in this work:
Questions: Is IS*256* transposition activity affected by low concentrations of antibiotics? Is IS*256* involved in generation of intermediate vancomycin resistance in *S. aureus* (VISA: vancomycin intermediate *S. aureus*)?
Methods: The transposition frequency of IS*256* was monitored using a recombinant IS element in several host strains in the presence and absence of antibiotics and in the absence and presence of Sigma factor B. Insertions of IS*256* were mapped by genomic sequencing in a clinical (SA137/93A) and a laboratory VISA isolate (SA137/93G).
Results: Low concentrations of antibiotics seem to activate transposition frequency whereas the activity of the stress sigma factor B inhibits the transposition of IS*256* (2, 3). Furthermore, we identified the 3' end of the *rsbU* gene, which encodes a positive regulator of sigma factor B, as a hotspot for IS*256* insertion in the clinical isolate *S. aureus* SA137/93G as well as in the laboratory strain *S. aureus* HG001. Interestingly, subinhibitory concentrations of chloramphenicol in combination with heat stress, as well as linezolid and spectinomycin at physiological temperatures, selected for such *rsbU*::IS*256* insertion mutants. In consequence of the inactivation of *rsbU*, the IS*256* transposition frequency was increased 4-fold in the *S. aureus* HG001 mutant (4). Sequencing showed that the two VISA strains contained the so far highest number (SA137/93A: 44 insertions; SA137/93G 38 insertion) of insertions of IS*256* of all sequenced strains harbouring this IS element. For two insertions an influence on resistance to vancomycin was demonstrated (5).
Conclusions: Low concentrations of antibiotics may activate transposition of IS*256*. As shown for the VISA strains the insertions may lead to an increase in resistance against antibiotics or - after insertion into a regulatory gene - an increase in transposition frequency.